

OPEN PEER REVIEW REPORT 1

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Title: Effects of mesenchymal stem cell-derived exosomes in neurogenesis and cognitive impairment of Alzheimer's disease model

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COMMENTS TO AUTHORS

Summary: In the manuscript entitled "Effects of mesenchymal stem cell-derived exosomes in neurogenesis and cognitive impairment of Alzheimer's disease model," the authors describe the effect of Mesenchymal Stem Cell-derived exosomes on cognitive and cellular function in an AD mouse model.

Overall, this is potentially a promising report presenting several sets of the novel and highly relevant results demonstrating that Mesenchymal Stem Cell-derived exosomes possess neuroprotective properties.

However, in its current form, this study has several major issues that should be carefully addressed.

Critiques:

General:

1. Introduction. Recent work by Mariana A. de Godoy et al. "Mesenchymal stem cells and cell-derived extracellular vesicles protect hippocampal neurons from oxidative stress and synapse damage induced by amyloid- β oligomers," which is highly related to the study should be referenced in the introduction section (de Godoy et al., 2018).

2. Methods.

a) Induction of AD and treatments administration. There is a problem with the method used to generate and treat AD mice. The authors injected the animals twice into the same region of the brain. In this particular case with an acute model of AD, I would apply chronic cannulation as an alternative for repeated acute injections (Faucher et al., 2016), or intracerebroventricular injection of Amyloid- β peptides and hippocampal injection of exosomes (or vice versa). Moreover, the coordinates have not been chosen correctly for the injections. According to the coordinates mentioned in the methods section, the injection site is outside of the dentate gyrus and even hippocampus!

Additionally, the researchers administered the mice bilaterally with 3 μ L of A β solution, which is an enormous amount for a single injection into the mice hippocampus (average volume is about 0.5-1 μ L). The injection rate of 0.5 μ L/min was very high as well. The regular recommended rate is about 0.1 μ L/min (Faucher et al., 2016). Furthermore, the authors did not check the quality and composition of their aged A β solution. It might be beneficial to run a western blot in order to show the composition of the oligomeric species.

b) Morris Water Maze test. The classic MWM paradigm includes the probe trial to measure how long

the animal spends in the "target quadrant" (the quadrant with the hidden platform). Please show. Moreover, a regular two-way ANOVA statistical analysis for MWM data was applied instead of repeated measures ANOVA.

- c) Immunofluorescence for DCX and PSA-NCAM markers. How many mice were sacrificed? How many brains were sectioned? How many sections per mouse were analyzed?
- d) There is no information about the image processing and analysis. What kind of piece of software was used to analyze the staining? N=? Surface area subjected to the analysis?
- e) Statistical analyses. The authors indicate that results are presented as means \pm SD even though all data are shown as mean \pm SE. Please explain.

3. Results.

- a) Please show aggregation of β A peptides by WB or another method.
- b) Novel Object Recognition (NOR) test results look very mistrustful! The authors state that the animals that were administered β A1-42 peptide aggregates demonstrated a lower discrimination index (figure 1B) when compared to the control group (30 % vs 50 % respectively). Moreover, the control animals (DI 50%) showed a preference for the novel object, on the contrary, animals that were administered β A 1-42 (ID 30%) did not seem to have the ability to differentiate between the familiar and the novel object. By definition, DI of 50% means no preference or no memory! DI less than 50% means novel object avoidance. DI of 30% means that the researchers cannot use NOR test to assess the memory acquisition in this particular model. Regularly, the wild-type control animals show DI of 60-70%. There are many articles, which could be used as references for this test. Look at the Stanford University official site, for example:

<https://med.stanford.edu/sbfnl/services/bm/lm/bml-novel.html>

- c) Administration of exosomes enhances neurogenesis in the SVZ. What does stereotaxic analyses mean? How the quantification of PSA-NCAM/DCX positive cells was performed. Area? Software? Threshold? N=?

- d) The quality of the images Fig. 3 is not adequate for publication in NNR.

Specific minor points:

1. I would suggest another title for the article.
2. You should explain each of your abbreviations the first time it appears in the main text. No abbreviations should be used in the Abstract.